

mercury and cadmium ions with high affinity are disclosed in Table 2. The chelon proteins can be within cells or on the surfaces of the cells in which they were produced, for example, for use in methods of concentrating heavy metal ions from a contaminated aqueous environment, or waste stream, or the chelon proteins can be immobilized onto a solid support for use in removal of heavy metal ions from a contaminated aqueous medium. The MerR protein can also be produced for *in situ* metal ion binding, or it can be purified and immobilized to a support material.

Also within the scope of the present invention are methods of removing heavy metal ions from contaminated aqueous solutions, waste streams or contaminated environments, for example, using immobilized chelon or MerR proteins, immobilized cells containing the MerR or chelon proteins or using whole plants to take up, sequester and concentrate the heavy metal ions from contaminated soil, ground water, hydroponic solutions, irrigation water or waste streams. Selective expression of the MerR or chelon proteins in above-ground plant parts (such as stems and leaves) allows the harvesting of the plant material in which the heavy metal ions have been sequestered and concentrated. Those plant parts can then be disposed of or processed in an environmentally appropriate manner.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C illustrate the full length wild-type MerR protein, as the monomeric metal binding domain, the dimeric coiled-coil metal binding domain and the mercury-bound dimeric metal binding domain, respectively. In each, the tube represents the coiled-coil region, the small open spheres represent cysteine residues, the hatched curve is the short loop regions, the large cross-hatched circles represent all other regions of the protein, and the small black sphere represents bound mercuric ion. Dimer in solution binds one Hg ion because of the conformational change. Figure 1B illustrates the chelon protein with its tandem metal binding domains. The shapes are as in Figure 1A except that the hatched curves represent the nonwild-type linker residues which allow association of the coiled-coils and stabilize the protein.

Figure 2 graphically illustrates the results of Hg(II) binding as measured by equilibrium ultrafiltration. The capacity of the chelon appears to be higher than that of the naturally occurring MerR protein.

Figure 3 provides the results of Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF, using a Bruker Reflex Instrument) of a purified mercury-specific chelon protein as eluted from a StrepTag column. Peaks shown are the single positive charge at 12,817.7 Da and the double positively charged species at 6412.0 Da. Both peaks are in reasonable agreement with the expected values for the chelon protein (12821.58 Da). Tailing on major peaks arises from random adducts of sinapinic acid from the matrix (224 Da).

Figure 4 summarized steps in the cloning of the chelon coding sequence into the pASK-IBA3 vector. In PCR reaction 1 (with primers corresponding to SEQ ID NO:13 and SEQ ID NO:14) and PCR reaction 2 (with primers corresponding to SEQ ID NO:15 and SEQ ID NO:16), each with pNH9 as template, the chelon coding sequence DNA fragment was synthesized. The two PCR products were digested with BsaI and BamHI and ligated into pASK IBA3 (Genosys) which had been cut with BsaI. The ligation products were transformed into *Escherichia coli* XL1-Blue and transformants were selected using ampicillin. In the resulting recombinant vector (pASK MBD, carrying the chelon of SEQ ID NO:4), chelon expression is induced by anhydrotetracycline. Tetracycline can also be used to induce expression. PNH9 has been described in Hamlett et al. (1992) *J. Bacteriol.* 174:6377-6385.

Figure 5 shows binding of  $^{203}\text{Hg}$  ions by cells expressing MerR or chelon protein (SEQ ID NO:2); the data were collected in two experiments.

## DETAILED DESCRIPTION OF THE INVENTION

In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given to such terms, the following definitions are provided.

A coding sequence is the part of a gene or nucleic acid molecule which codes for the amino acid sequence of a protein, or for a functional RNA such as a tRNA or rRNA.

Complement or complementary sequence means a sequence of nucleotides which forms a hydrogen-bonded duplex with another sequence of nucleotides according to Watson-Crick base-pairing rules. For example, the complementary base sequence for 5'-AAGGCT-3' is 3'-TTCCGA-5'.

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Downstream of means on the 3' side of any site in DNA or RNA, and upstream of means on the 5' side of a site in DNA or RNA.

Expression refers to the transcription of a coding sequence into structural RNA (rRNA, tRNA) or messenger RNA (mRNA) and subsequent translation of a mRNA into a protein.

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An amino acid sequence that is functionally equivalent to a chelon or MerR protein of the present invention is an amino acid sequence that has been modified by single or multiple amino acid substitutions, by addition and/or deletion of amino acids, or where one or more amino acids have been chemically modified, but which nevertheless retains the heavy metal binding activity of a chelon (or MerR protein) of the present invention. Functionally equivalent nucleotide sequences are those that encode polypeptides having substantially the same biological activity.

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Two nucleic acid sequences are heterologous to one another if the sequences are derived from separate organisms, whether or not such organisms are of different species, as long as the sequences do not naturally occur together in the same arrangement in the same organism.

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Homology refers to the extent of identity between two nucleotide or amino acid sequences.

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Isolated means altered by the hand of man from the natural state. If an isolated composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not isolated, but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is isolated, as the term is employed herein.

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